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ORIGINAL ARTICLE

Real-time telemetric monitoring in whole-body ^{60}Co gamma-photon irradiated rhesus macaques (*Macaca mulatta*)

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Keywords
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Abstract

Background Animals undergoing experimental manipulations, such as exposure to radiation, may exhibit physiologic and behavioral signs of pain and distress. Telemetry permits close monitoring of these parameters for early and effective management during procedures.
Methods Radiotelemetric units were surgically implanted into 24 *Macaca mulatta* before 6.5-Gy cobalt-60 γ-photon irradiation. Each unit transmitted electrocardiogram, intrathoracic pressure, and body temperature leads. Primate irradiation-restraint boxes and housing cages were modified to collect telemetric signals before, during, and after irradiation.
Results Differences in respiratory rate, heart rate, or body temperature in telemetric-collected recordings, which were observed during non-irradiation and irradiation sessions, were statistically insignificant.
Conclusions Insignificant changes in the physiological parameters during monitoring suggest that the animals experienced no detectable pain or distress during irradiation.

Introduction

Rhesus macaques (*Macaca mulatta*) have been used as non-human primate (NHP) models for a number of stress-related situations [1, 8, 9, 11, 16, 17, 21]. A key aspect of United States (US) animal welfare regulations [18, 19] is that pain, distress, and discomfort should be minimized whenever possible.

The refinement of our experimental procedures is an important technique to improve animal welfare. Although significant improvements have been made to research protocols to reduce, refine, and replace animals in experiments, one area of research where the use of animals continues is in whole-body irradiation.

Here, we examined current NHP irradiation procedures used at the Armed Forces Radiobiology Research Institute (AFRRI), using radiotelemetry implants to improve recognition and alleviation of pain, distress, or discomfort. This close monitoring technique was used to improve our experimental designs to accommodate animals' needs by preventing or ameliorating pain. An additional goal was to identify practical endpoints to determine when a study design should be changed or a study should be ended early.

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14. ABSTRACT
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The International Association for the Study of Pain defined pain in humans as 'an unpleasant sensory and emotional experience associated with potential or actual tissue damage, or described in such terms' [6]. Pain can be divided into four categories: momentary pain, post-procedural/post-surgical pain, persistent pain, and chronic pain [7]. While the ability of animals to feel pain is poorly understood, scientists and ethicists believe that animals perceive pain in ways very similar to those of humans and, according to US government principles, 'unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals' [20].

Distress is even more difficult to define in experimental animals. The current accepted definition of stress is the biological responses that an animal exhibits in an attempt to cope with a threat to its homeostasis [6]. When stressors are mild or of short duration, the animal may regain homeostasis without any lasting effects. Stress results in distress to the animal when the stressor disrupts biological functions that are critical to the animal's well-being. When normal function is disrupted, pathology may occur, threatening the animal's welfare and causing distress.

The US Department of Agriculture (USDA) Animal Care Policy Manual, Policy 11, Painful Procedures [18] uses studies involving irradiation as examples of a procedure that can be expected to result in more than momentary or slight pain and distress although the radiation itself does not cause pain or distress directly. Pain and distress result from the sequelae of tissue injury following acutely delivered high whole-body ionizing radiation doses that result in hematopoietic failure. The severity of radiation illness depends not only on the total dose received but also on other factors such as the areas of the body exposed, gender, age, physical condition of the animal, and husbandry provided. It is well known that radiation dose responsiveness (i.e., relative susceptibility and resistance) varies among animal species and strains [2, 3].

Previously, pain, distress, and discomfort in animals during acutely delivered whole-body irradiation in a high-level radiation area could be monitored only remotely by video and audio equipment. In this study, we addressed whether changes in telemetrically measured physiologic signs—respiratory rate, heart rate, and body temperature—could be used to detect indicators of pain, distress, and discomfort during irradiation and to detect signs of radiation-induced disease earlier than possible with conventional clinical observations and, thereby, facilitate earlier treatment to improve outcome. Telemetric transponders surgically implanted in the animals monitored physiologic parameters remotely via a system that recorded real-time measurements in the control room of the AFRRI γ-photon irradiation facility. To our knowledge, this was the first real-time, simultaneous recording of the respiratory rate, heart rate, and body temperature of conscious NHPs before, during, and after whole-body irradiation.

Materials and methods

Subjects

Twenty-four clinically healthy adult male rhesus macaques (*M. mulatta*), 7–13 kg and 5–14 years of age, were obtained from the non-naïve pool of NHPs of the US Department of Defense. The NHP colony was considered a heterogeneous resident population, and individuals were not segregated by age or prior study status. However, only males were used to reduce variability. All animals previously had participated in *Helicobacter pylori*, malaria, and/or dengue fever protocols. Ten animals were of Chinese origin and 14 were of Indian origin [4]. All were housed individually indoors at the AFRRI facility, which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, in 6.1-ft^2 (0.57-m^2) stainless steel primate cages. Environmental conditions, including temperature, humidity, and lighting, were in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals*. All cages were equipped with built-in perches, foraging boards, stainless steel mirrors, and other manipulanda. Animals were fed Harlan 2050 Teklad Global 20% primate diet (Harlan Teklad, Madison, WI, USA) twice daily, supplemented with fresh fruit and vegetables, and received autoclaved water *ad libitum*. The experimental protocol was reviewed and approved by the AFRRI Institutional Animal Care and Use Committee and received second-tier independent veterinary-level approval.

Irradiation

All animals were subjected to the following behavior-modifying activities for the irradiation procedures. They were transported in rolling, covered transport containers on the day of irradiation to a 35-ft by 35-ft by 25-ft ($10.7 \times 10.7 \times 7.6$ m = 870 m^2) room that contained a ^{60}Co γ-photon irradiation facility. The room was maintained at 68°F (20°C) and contained facilities for maintenance of the radiation unit. Animals were divided into six 'cohorts' of four monkeys. Irradiation of cohorts was performed during the course

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of several months to accommodate the post-irradiation care of the animals and collection of specimens at timed intervals. Each animal served as its own control for comparing data before and after irradiation. All animals in a cohort were irradiated individually on the same day, one cohort per month, with each animal in an acrylic restraint box (Fig. 1). Once the animal and restraint box were secured, 6.5-Gy ^{60}Co γ-photon radiation was administered at 0.4 Gy/minute, measured at the midpoint of the abdomen. Dosimetry was performed using a water-filled plastic cylinder matched to the body mass and girth of each animal as previously described [14]. Radiation dose and dose rate were measured using an alanine pellet system that was calibrated to standards from the National Institute of Standards and Technology in Gaithersburg, MD, USA, and from the National Physics Laboratory in the UK.

Fig. 1 Acrylic plastic restraint box used for radiation field mapping and irradiation of non-human primates. Slotted sides for adjusting the body support 'seat' accommodated the subject's height. The first groove was placed at a height of 53.1 cm, while the second through the sixth grooves were placed at 4-cm intervals. Four knobbed plastic screws can be used to adjust and tighten the seat from front to back. A grooved neck plate at the top of the restraint box is adjustable to fit different-sized collars. Adjustments of the body and neck supports allowed the animals to fit comfortably in the restraint box. Six black nylon t-bar cleats were used to secure the animal's extremities with 3/8-in nylon rope. Fabric strips were used to prevent chafing on arms and legs. White cords on each side aided in lifting the box. A vertical sliding door (not shown) was slid into place to make dosimetric determinations, to secure the animal within the box during irradiation, and to provide stability to the box.

Expected post-irradiation signs of pain and distress and alleviating treatments

Historically, in our vivarium, monkeys were provided care in the following manner for adverse clinical signs after irradiation. Depending upon the irradiation dose, on days 0–5, monkeys could exhibit nausea, vomiting, anorexia, lethargy, diarrhea, fever, moist desquamation of the mucous membranes, erythema, or edema. Further, decreased numbers of white blood cells, lymphopenia within 24–48 hours, and neutropenia are likely to occur. During this period for these clinical signs, the monkeys could be treated with oral electrolytes *per os (p.o.)* daily. The rooms could be completely sanitized weekly, and the cages washed thoroughly three times a day; the food could be sterilized, and the monkeys could be offered moist appealing foods as circumstances required. During days 5–8, fever, gastrointestinal ulceration, and bloody diarrhea could occur. Blood pressure, thoracic pressure, and blood albumin concentration could decrease, and the hematocrit and temperature could increase. Basic clinical supportive care would be provided. On days 8–14, bleeding, anemia, and thrombocytopenia could occur. Moistened biscuits with fruit juice, fluids, and/or colloids would be provided. During days 14–25, the progression of clinical signs could continue, clinical signs could resolve, and minor hair loss or blistering of skin and mucous membranes could occur.

Telemetric devices

Radiotelemetric devices (Konigsberg telemetry implant Model T30F, Konigsberg Instruments, Inc., Pasadena, CA, provided by Integrated Telemetry Systems (ITS), Inc., Pinckney, MI, USA) were sterilized by ethylene oxide before implantation. Microbiologic evaluation of the implants revealed that sterilization was complete within the limits of such testing. Telemetric devices could be turned on and off externally to conserve power. In addition, before implantation and use, we demonstrated that the devices and their function could withstand the γ-photon dose used to irradiate the animals. The frequency-modulated (FM) radio signals produced by the implants were transmitted from

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within the γ-photon field during irradiation and received without interference in the radiation preparation area. In short, we established that the implant devices were operable subsequent to both ethylene oxide sterilization and the dose of radiation used in this study.

Surgical procedure for telemetric transponder implantation

A radiotelemetric transponder was implanted into each animal according to the manufacturer's detailed instructions (ITS, Remote Monitoring Integrated Scientific Systems, R.M.I.S.S., Inc., Wilmington, DE, USA). The surgical procedure and the telemetry system have been described in general [4, 5]. The animals were sedated initially with ketamine hydrochloride (Ketaset, Fort Dodge Animal Health, Overland Park, KS, USA), administered intramuscularly at 10 mg/kg of body weight. Each was endotracheally intubated and maintained under isoflurane (1–3%) anesthesia for the duration of the procedure. Two surgical sites were prepared. The first incision was in the thorax (dorsally, four intercostal spaces cranial from the caudal extant of the ribcage) for implantation of the electrocardiogram (ECG) and intrathoracic pressure (ITP) sensors. A second incision, for the implant module, was placed intramuscularly in an abdominal subcutaneous pocket created in the left flank region just below the diaphragm, midway between the spine and the sternum. Sensor wires (i.e., leads) for the ECG, ITP, and temperature (T) were tunneled subcutaneously to their specified locations (Fig. 2). In addition, a transmittal antenna was routed from the implant module body near the spine toward the head.

Buprenorphine (0.01–0.05 mg/kg) was administered intramuscularly to the animals for 2–3 days at 6- to 12-hour intervals after surgery and thereafter as required. Also, standard pre-operative and post-operative antimicrobial therapy with cefazolin (Sussex Drug Products Company, Edison, NJ, USA) was given at 25 mg/kg intramuscularly or *p. o.* The animals were closely monitored daily for approximately 10 days. They were allowed to recover for at least 30 days after surgery before assignment to the study and irradiation procedures. No infections were noted as determined by daily evaluations of the surgical incisions.

Telemetry system design and setup

Data, transmitted via radiotelemetry, were recorded by CA Recorder™ systems and VR^2 software (Data Integrated Scientific Systems (D.I.S.S.), Dexter, MI, USA)

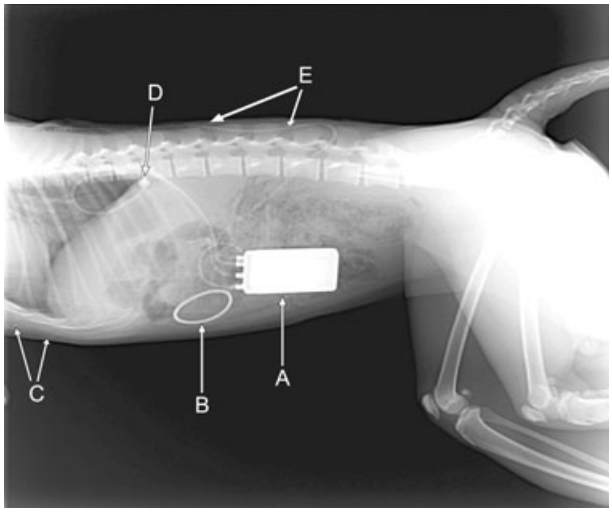

Fig. 2 Radiograph of a T30F radiotelemetric implant in a non-human primate. Shown are a battery (A), with appropriately placed electrocardiogram leads (C), and the locations of an intrathoracic pressure transducer (D) and antennae (B, E) [faint image]. The implant is placed in a pouch in the lateral abdominal musculature. The circular antenna (B) is used to turn the implant on and off externally to conserve battery life.

[9]. FM radio signals for ECG, ITP, and T were received by a single-pole antenna mounted inside the modified perch of each animal cage. The perch was modified by placing the recording antenna in a polyvinyl chloride pipe. This is an ideal location because most NHPs spend the majority of their time on their perches [15]. Cage bars were cut on the cage front to allow for insertion of the antennae into the tube/perch. Also, acrylic plastic sheets were installed on the cage tops to prevent the animals from reaching and damaging the cables running from the cages to the wall and into the adjacent radiotelemetric control room.

The telemetric signals, carried by coaxial cable into the control room, were demodulated at the ITS base station (Fig. 3). The signals were then digitized, captured, and recorded on the workstation hard drive where they were processed by the CA Recorder™. ITP, ECG, and T for each animal were recorded simultaneously and continuously at 1-minute intervals, with 4-minute averages, for each parameter. Sets of four animals were monitored simultaneously daily for 5–7 days before irradiation and 25 days after irradiation (a total average of 768 hours/animal) in each of two dedicated rooms using two base stations. Recorders were turned off each day while rooms and cages were cleaned. No acclimation or training was required because the animals were remotely monitored in telemetry-fitted cages similar to those in their standard housing environment.

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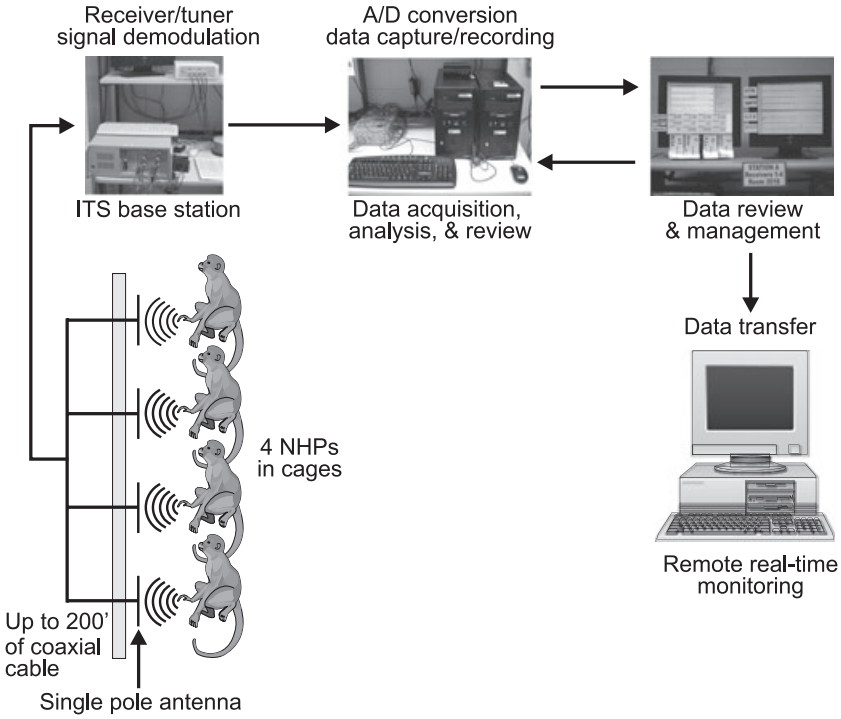

Fig. 3 Schematic diagram of the radiotelemetric monitoring system components. The system can monitor four non-human primates per workstation. Frequency-modulated radio signals were received by single-pole antennae within each primate cage. The signal was carried by coaxial cable to the control room and into a receiver/tuner where it was demodulated at the integrated telemetry systems base station. The signal was then converted to a digital signal, captured and recorded on the workstation hard drive, and processed by CA Recorder™ and VR^2 data review and management software. Real-time monitoring was conducted at the remote workstation.

In addition to the control room base stations, a portable telemetric recording station was brought into the γ-photon irradiation control and preparation room for the purpose of measuring, in real time, the heart rate, respiratory rate, and body temperature during non-irradiation and acutely delivered whole-body irradiation sessions. An unshielded recording antenna was positioned on one side of the restraint box in the γ-photon irradiation facility.

Acclimation

Animal distress reactions may occur as a result of unfamiliarity with experimental procedures and staff, separation from other NHPs, or the sights, smells, and sounds of equipment in a strange room. Thus, several weeks before irradiation in three training periods a week apart, the NHPs were acclimatized to experimental handling procedures in the following manner. They were fitted and acclimated to catch collars (Primate Products, Inc., Immokalee, FL, USA) for safe ease of manipulation from their home cages to restraint cages where physical examinations were performed. Whole-body radiation exposure requires that animals be stationary for extended time periods for accurate dose delivery. To reduce variation in total radiation dose delivery, each animal was lightly sedated with 5–8 mg of ketamine hydrochloride and positioned in a specially designed restraint box (Fig. 1) the same way, with the arms moved upward and forward so that they did not shield the torso. Placement in the restraint box took place in the vivarium. The restraint devices were used to provide safe, efficient transport between the γ-irradiation facility and the vivarium and to position the primates appropriately for irradiation without the use of anesthetics. The total time of restraint was estimated to be 40 minutes or less and was sufficient to accomplish the objectives. Some animals were previously trained for acceptance to chair restraint, while other untrained animals were gradually acclimated to the clear Plexiglas restraint devices in training sessions for up to 30 minutes each. Subsequently, the animals were transported in special enclosed, rolling transport containers to the irradiation facility. The animals were allowed to recover from sedation before a 15-minute non-irradiation period, followed by a 5-minute intermission and a 15-minute irradiation period. Thus, all subjects were acquainted with the entire procedure of catching, sedating, loading into the irradiation-restraint boxes, transporting to the irradiation facility, being carried into the exposure room, and positioning within the room. Telemetry parameters were recorded while the animals were in the irradiation facility. When recording was completed, the monkeys were returned to their home cages in the vivarium. The

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data collected during the non-irradiation and irradiation periods were compared.

Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM). GRAPHPAD PRISM® 3.0 (GraphPad Software, Inc., La Jolla, CA, USA) statistical analysis, one-way ANOVA, two-way ANOVA, and Dunnett's multiple comparison test were used to compare groups, with 5% as a significant level.

Results

Implant performance

This study was performed over a 1-year period during which some of the implants lost signaling capability, resulting in fewer animals for evaluation of telemetric data as noted.

Respiratory rate

Of the 24 implanted animals, only 11 ITP lead signals retained sufficient quality to compare respiratory rates for the non-irradiation and irradiation recording sessions (Fig. 4A). Differences in respiratory rates among the 11 animals before the irradiation sessions (P = 0.9994, df = 14, F = 0.1927) or during the irradiation sessions (P = 0.9999, df = 14, F = 0.152) were statistically insignificant, and differences between the non-irradiation and irradiation sets of data were statistically insignificant (P = 0.9999, df = 29, F = 0.2794).

Heart rate

Of the 24 implanted animals, the heart rate data of only nine irradiated animals could be compared to the data from their non-irradiation recording sessions. In these animals, there was some variation and an apparent slight increase in the heart rate during the non-irradiation session (Fig. 4B). The heart rates started at about 190 ± 5 beats/minute (bpm) and ended near 210 bpm, whereas during irradiation, heart rates were approximately 220 ± 5 bpm. By one-way ANOVA, differences in heart rates among the nine animals for a 15-minute period before the irradiation sessions (P = 0.2412, df = 14, F = 1.265) or during the 15-minute irradiation sessions (P = 1, df = 14, F = 0.08792) were statistically insignificant, and differences between the non-irradiation and irradiation sets of data were statistically insignificant (P = 0.6513, df = 30, F = 0.8794).

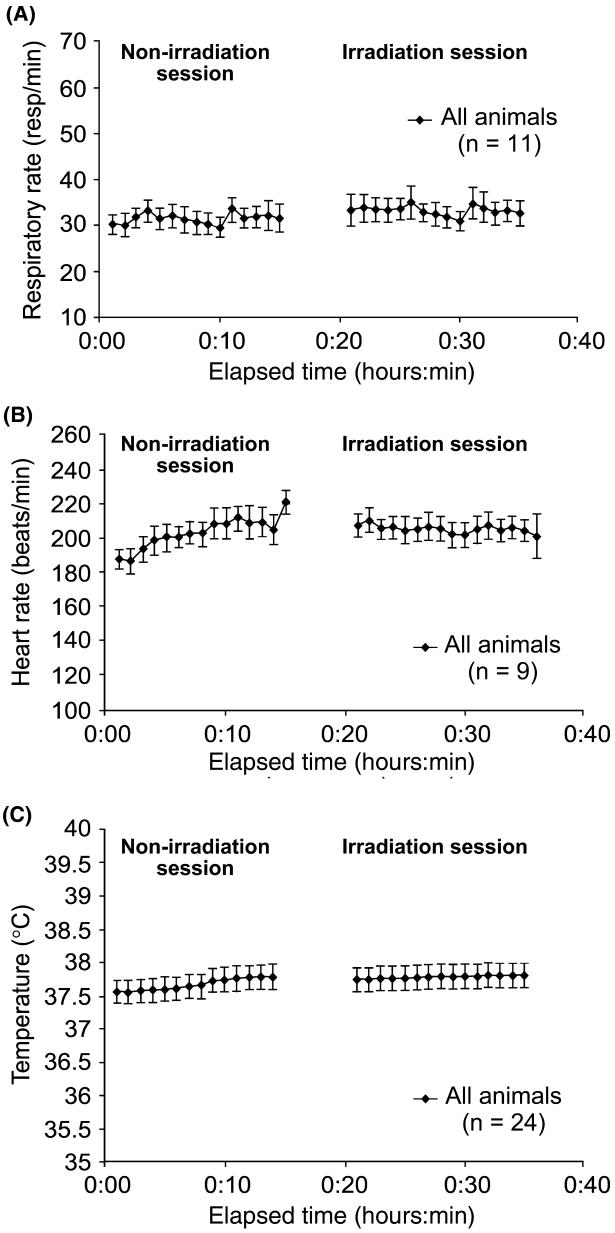

Fig. 4 Mean respiratory rate, heart rate, and body temperature. Data reflect a 15-minute non-irradiation period, a 5-minute interval without data collection, and a 15-minute irradiation period. (A) The mean (±SEM) respiratory rate of 11 non-human primates. (B) The mean (±SEM) heart rate of nine animals. (C) The mean (±SEM) temperature (°C) of 24 animals. The total number of animals representing each variable differs because of the failure of some implants to transmit signals for respiratory rate and/or heart rate at the time of irradiation.

Temperature

Body temperature recordings for all 24 of the irradiated animals are presented and are compared to the

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recordings for their non-irradiation sessions (Fig. 4C). Differences in temperatures among the 24 animals before the irradiation sessions ($P = 0.982$, df = 14, F = 0.3712) or during the irradiation sessions ($P = 1$, df = 14, F = 0.01935) were statistically insignificant, and differences between the non-irradiation and irradiation sets of data were statistically insignificant ($P = 1$, df = 29, F = 0.2691).

Discussion

This study found no significant changes in the subjects' respiratory rate, heart rate, or body temperature during non-irradiation and ^{60}Co γ-photon irradiation sessions. The finding suggests that the animals experienced no additional pain, distress, or significant discomfort during the process of γ-photon irradiation delivered to the total dose and dose rate in this study.

The respiratory rates of the 11 animals recorded during both sessions were within the normal range for awake rhesus macaques of 35–50 breaths/minute [2, 10] and were comparable with rates before and after the sessions, remaining relatively steady at about 33 breaths/minute. A heart rate for an awake adult rhesus macaque, when measured by telemetry, is 98–122 bpm [2, 12]. Others report higher ranges in awake rhesus macaques of 150–333 bpm [2, 13], which would be more in line with our observations. Thus, during both non-irradiation and irradiation sessions, there was some apparent elevation above normal range but within the range reported by others [2, 13]. This apparent elevation may be explained by the fact that, despite previous acclimatization to irradiation procedures, the animals were slightly distressed by their unfamiliar environment compared to that of their home cages. During both sessions and between the two sessions, temperatures were 37–38°C (98.6–100.4°F), the normal range for rhesus macaques [10]. Despite attempts to acclimate the animals to the entire procedure, some apparent but statistically insignificant increases, compared to normal values, were noted in heart rate and temperature in both groups. Recovery from light sedation with ketamine may be one explanation for these changes.

A likely explanation for the loss of signal in some implanted units is that the battery power for implanted units was expended because of the extensive post-irradiation recording period. According to the manufacturer, the estimated battery life for the implants is 6 months to 1 year, depending on the total amount of time the system is queried for data output. We noted two other possible reasons for signal loss. First, in some animals the sensor lead was not in an ideal location, and tissue fibrosis may have interfered with the functioning of the ITP sensor for respiratory rate recordings. Second, in some of the larger animals, the sensor cable was of insufficient length to remain in optimal position during natural movement.

Assessment of pain and distress in animals is a complex task especially in an irradiation facility. In previous work with NHPs subjected to irradiation procedures, the animals were monitored continuously by audio and video monitors. In this study, telemetry was used and evaluated because it provides direct physiologic measures, indicating early signs of clinical changes and, perhaps, even signs of discomfort, distress, or pain. In addition, telemetric monitoring enables investigators to detect important cardiopulmonary responses to treatments early and allows intervention sooner than without telemetry. For example, treatments could be initiated or changed to optimize outcomes particularly regarding radiation illness and/or infection. Telemetry would improve development of potential therapeutic protocols with animal models.

It is noteworthy that none of the telemetry devices malfunctioned or had long-term damage as a direct result of the radiation exposure. Testing prior to surgical implantation indicated that the devices and their functionality were not affected by the radiation dose used in this study. Failures to send signals were related primarily to decline of the battery power in each unit, which had been activated by the manufacturer up to 1 year before the irradiation. Hence, the units should be activated only immediately before implantation. Loss of signal quality from several of the implanted telemetry devices was due partially to placement of the device. In some of the larger, older animals, the standard-sized sensor leads were too short and had a tendency to change position during normal animal movement. This finding led the manufacturer to realize that a single length of sensor leads is not adequate in all animals. Replacement of malfunctioning leads, which became embedded in fibrotic tissue, was not attempted in any of the animals because telemetry was just a portion of a broader study that required adherence to the experimental research schedule. As the animal implant model is further developed and refined and as the surgical technique is mastered, fewer complications would be expected.

It is our understanding that this is the first reported use of telemetry for assessing changes in detectable physiologic parameters for γ-photon irradiated rhesus macaques. This study clearly demonstrates the feasibility of using telemetry recording during acutely delivered whole-body γ-photon irradiation, and it finds no significant changes in body temperature, heart rate,

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and respiration rate during such irradiation. It remains to be determined whether different qualities of radiation (e.g., neutrons and protons) and variations in total doses and dose rates would permit or inhibit measurements of the fundamental parameters presented in this report.

In conclusion, telemetric data collection may be useful in studies designed to evaluate physiologic changes during delivery of radiation and subsequent radiation illness as well as the prompt timing and effectiveness of care and intervention for managing the sequelae of radiation-induced sickness. Radiotelemetry could be an appropriate adjunctive measure in future studies to fully examine, compare, and select the best radiation countermeasures as well as other physiologic disturbing agents.

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